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DNA chimerism and its consequences after allogeneic hematopoietic cell transplantation

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The unphysiological formation of biological chimeras after allogeneic hematopoietic cell transplantation is not free of consequences. Recent findings suggest that in the transplant recipient some epithelial cells reveal, unexpectedly, donor-derived genotype and/or acquire genomic alterations. Since both phenomena are presented in the host epithelium, one could argue that they might be etiologically linked through a common background mechanism. We recently proposed that the incessant charge of the transplant recipient with donor-DNA and its integration in host epithelium by horizontal DNA transference may indeed be operative in the generation of epithelial cells with donor derived genome. On the other hand, the incessant incorporation of the foreign DNA into the host genome may result in genomic alterations. Lymphocyte-epithelial interactions between the two genetically distinct cell populations in the transplant recipient should be investigated more precisely not only in cellular but also in molecular level.

Key words: hematopoietic cell transplantation, chimerism, horizontal gene transfer, genomic instability, secondary cancer

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Allogeneic hematopoietic cell transplantation (allo-HCT) in humans results in true biological chimeras. While circulating hematopoietic cells and their tissue derivatives (e.g. Langerhans cells) become donor genotype after transplantation, other cells remain recipient in origin. This unphysiological formation of biological chimeras is not free of consequences. Recent findings of our group and others have shown that, besides Graft versus Host Disease (GvHD), there are also other consequences in the co-existence of two genetically distinct populations in the transplant recipient. First, epithelial cells with donor-derived genotype emerge,[1-2] a phenomenon, which was initially misinterpreted and falsely described as "stem cell plasticity". Second, epithelial tissues of the host acquire genomic alterations.[3]

Is chimerism in epithelium after allo-HCT, namely the emergence of distinct epithelial cells containing donor-derived genome, a real phenomenon or solely a technical artefact? Despite the initial scepticism and the methodological limitations on the detection of donor-derived non-hematopoietic cells in the transplant recipient, more recent studies of our group and others using strict criteria and examinations of isolated single cells clearly confirmed that following allo-HCT in humans, epithelial cells with donor-derived genotype emerge.[4-5] How does epithelial chimerism after allo-HCT occur? The mechanisms underlying this phenomenon remain unclear and divergent. Suggested mechanisms attempting to explain epithelial chimeric events after allo-HCT include transdifferentiation of hematopoietic cells into epithelial cells, generation of epithelial cells from unknown epithelial precursors and/or universal stem cells in the graft, and fusion of donor hematopoietic cells with recipient epithelial cells.[6-9] More recent findings suggest molecule trafficking as a novel mechanism of epithelial chimerism after allo-HCT. Jang et al[10] found that when murine hematopoietic stem cells are co-cultured with injured liver separated by a barrier, they may convert into liver-like

cells. Aliotta et al[11] and Ratajczak J et al[12] showed that this phenotypical conversion may be due to mRNA transfer between cells, resulting in an aberrant expression of foreign proteins in the recipient hematopoietic cells. However, such an mRNA transfer from epithelial to hematopoietic cells cannot explain recent findings of our group and others after clinical transplantation. First, Y-chromosome positive epithelial-like cells found in female allotransplant recipients were negative for expression of hematopoietic markers. Second, large amount of donor-DNA has been detected in blood-free fingernails obtained from transplanted recipients.[13] We also evaluated by quantitative microsatellite analysis the amount of donor DNA in 176 buccal swabs obtained from 71 patients after allogeneic transplantation and we found a high amount of donor-DNA (mean 26.6%) in the majority (89.7%) of them although no donor hematopoietic cells were evident in the samples by immunofluorescence.[14]

We recently proposed horizontal DNA transference as an alternative explanation for epithelial chimerism after allo-HCT.[14] Production of donor cells from the engrafted bone marrow is an ongoing process in the allo-transplanted recipient. Apoptosis is a well-recognized source of DNA in several clinical settings, such as cancer, extensive burning, GvHD and transplantation.[15-20] Donor cells undergoing apoptosis release donor-DNA packaged into apoptotic bodies.[21] Although foreign DNA is normally cleared up,[22] the fate of the large amount of released donor-derived genetic material in the transplant recipient is unknown. In an in vitro co-culture system mimicking the lymphocyte-epithelial interaction we showed that DNA can be horizontally transferred from apoptotic hematopoietic cells to the cytoplasm and nucleus of epithelial cell lines through phagocytosis of apoptotic bodies.[14] Both lysosomal inhibition in epithelial cells and repetitive load with apoptotic bodies, which may lead to saturation of lysosomal activity, increased the intercellular and intranuclear DNA delivery. The incessant charge of the transplant recipient with donor-DNA obtained from the engrafted bone marrow and its illegitimate integration in host epithelium by horizontal gene transfer may indeed be operative in the generation of epithelial cells with donor derived genome in transplant recipients.

Horizontal gene transfer is well described in prokaryotic organisms as a mechanism for functional and phenotypic change in order to adapt to different enviromental conditions. Horizontal gene transfer has also been shown in eukaryotic organisms both in vitro and in vivo. In vitro, the group of Holmgren has demonstrated that genomic DNA can be transferred to phagocytosing cells via the uptake of apoptotic cells.[23-24] In vivo, large fragments of plasmid DNA fed to mice passed into the bloodstream and integrated into the nucleus of white blood cells, spleen and liver cells.[25, 26] A critical role of DNA trafficking between cells has been also suggested in different clinical settings as in inflammation, in cancer and after organ transplantation.[17, 18, 27, 28] Most recently, Ehnfors et al[29] used a rat-to-SCID mouse tumor xenotransplant system and showed that tumor DNA can be transferred in vivo by detecting rat and mouse fusion chromosomes in mouse tumor stromal cells. Such an approach could be also applicable in a hematopoietic cell xenotransplantation system (e.g. human-to-mouse) where the detection of human-mouse fusion chromosomes would show that horizontal gene transfer can be operational in the context of allo-HCT.

After allo-HCT, epithelial tissues become injured through the preparative regimen and are then potentially attacked by allo-reactive T cells. The net effect of these alloantigeneic reactions is tissue stress and apoptosis, which we recognize clinically as GvHD. We hypothesized that chronic tissue stress due to interaction of donor-derived lymphocytes with host epithelium in the biological chimeras developed after HCT may cause genomic alterations. Indeed, our group and others found frequent genomic alterations measured as microsatellite instability (MSI, an established indicator of general genomic instability) in non-neoplastic epithelial tissues of patients who underwent allo-HCT, but not in control subjects including healthy individuals, autologous transplanted patients and patients before allo-HCT.[3, 30] Analysis of clinical data suggested the pivotal role of GvHD in the occurrence of MSI after allo HCT.[31] In an in vitro mutation analysis system we found that allostimulated Mixed Lympocyte Cultures may induce MSI in co-cultured epithelial cells.[31]

The development of epithelial cells with donor-derived genotype and the accumulation of genomic alterations in the epithelial tissues are only two of these recently recognized phenomena occurring in the chimeric organisms after allogeneic HCT. Since both phenomena are presented in the host epithelium, one could argue that they might be etiologically linked through a common background mechanism. A possible link between these two phenomena could be provided by horizontal gene transfer.

Taken together, current research results could support the following scenario after allo-HCT (**Figure 1**): the engrafted bone marrow produces continuously hematopoietic cells which after their programmed death charge constantly the host environment with donor-derived apoptotic bodies. In this context, the excessive amount of foreign material taken up repetitively by the recipient's professional and non-professional phagocytic cells may overwhelm their lysosomal capacity and thus part of the donor-derived apoptotic DNA fragments may escape degradation in the cytoplasm, be transferred into the nucleus and integrate within the recipient genome (DNA chimerism). This phenomenon may be more pronounced in GvHD lesions where the generated reactive oxygen species (ROS) from the activated lymphocytes may destabilize and damage lysosomes. Furthermore, the incorporation of the foreign DNA into the host genome could result in physical rearrangements at the site of integration, including point mutations, deletions, interruptions of coding sequences and chromosomal breakages. This "inappropriate" illegitimate integration of donor DNA in epithelial cells after allogeneic HCT may come in light as detection of epithelial cells with donor-derived genotype or as genomic instability in the epithelium and may have implications in the development of secondary cancers.

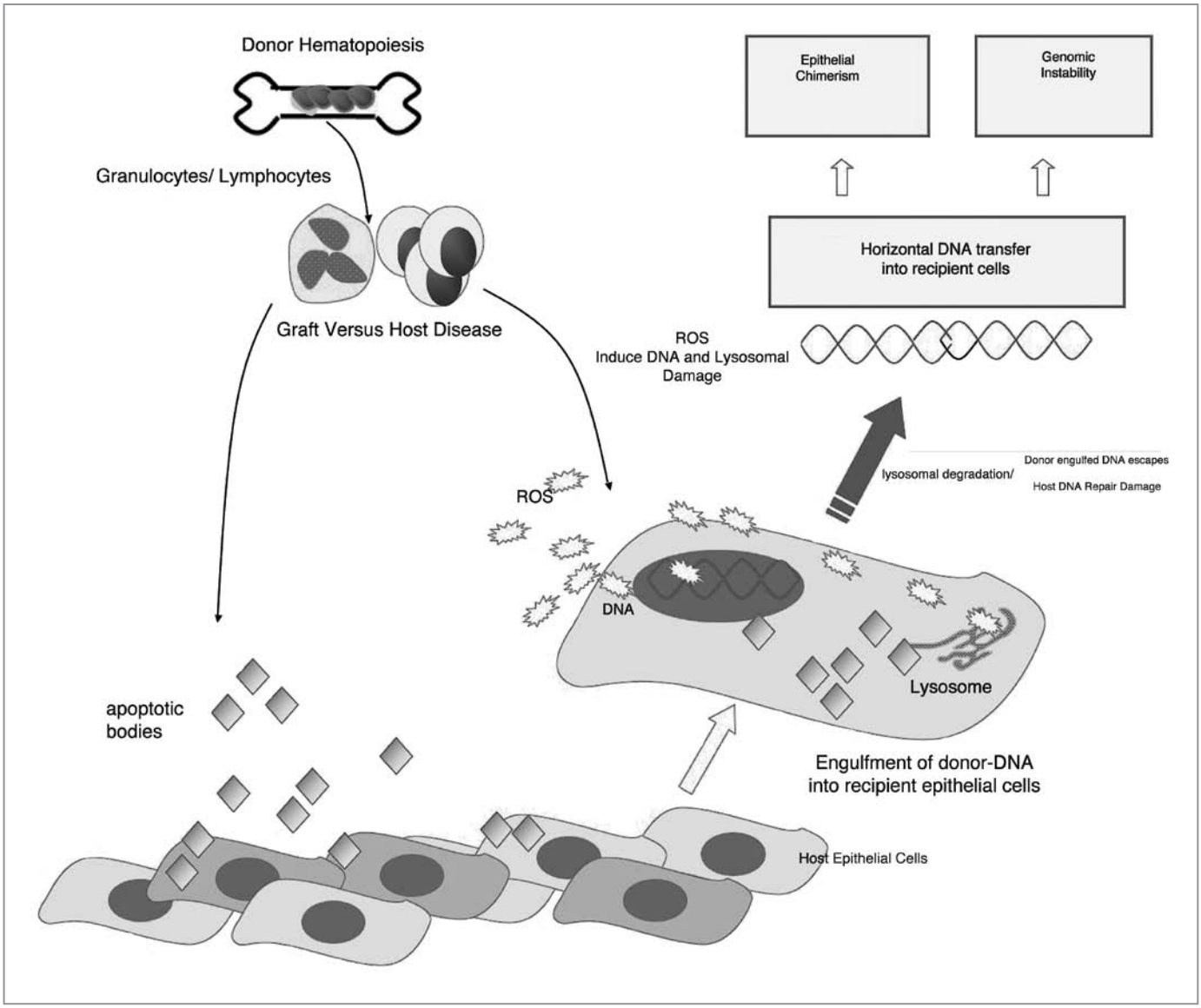

Figure 1. A proposed model of DNA Chimerism through Horizontal Gene Transfer and its consequences after allogeneic hematopoietic cell transplantation (see text).

References

1. Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R et al. Multi-organ, multilineage engraftment by a single bone marrow-derived stem cell. Cell 2001;105: 369-77.
2. Metaxas Y, Zeiser R, Schmitt-Graeff A, Waterhouse M, Faber P, Follo M et al. Human hematopoietic cell transplantation results in generation of donor-derived epithelial cells. Leukemia 2005;19: 1287-9.
3. Faber P, Fisch P, Waterhouse M, Schmitt-Gräff A, Bertz H, Finke J et al. Frequent genomic alterations in epithelium measured by microsatellite instability following allogeneic hematopoietic cell transplantation in humans. Blood 2006;107: 3389-96.
4. Spyridonidis A, Schmitt-Gräff A, Tomann T, Dwenger A, Follo M, Behringer D et al. Epithelial tissue chimerism after human hematopoietic cell transplantation is a real phenomenon. Am J Pathol 2004;164: 1147-55.
5. Tran SD, Pillemer SR, Dutra A, Barrett AJ, Brownstein MJ, Key S et al. Differentiation of human bone marrow-derived cells into buccal epithelial cells in vivo: a molecular analytical study. Lancet 2003; 361(9363): 1084-8.
6. Spyridonidis A, Zeiser R, Follo M, Metaxas Y, Finke J et al. Stem cell plasticity: the debate begins to clarify. Stem Cell Rev 2005;1: 37-43.
7. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418(6893): 41-9.
8. Kucia M, Halasa M, Wysoczynski M, Baskiewicz-Masiuk M, Moldenhawer S et al. Morphological and molecular characterization of novel population of CXCR4+ SSEA-4+ Oct-4+ very small embryonic-like cells purified from human cord blood: preliminary report. Leukemia 2007;21(2): 297-303.
9. Kucia M, Halasa M, Wysoczynski M, Baskiewicz-Masiuk M, Moldenhawer S, Zuba-Surma E et al. Transplanted bone marrow regenerates liver by cell fusion. Nature 2003;422(6934): 901-4.
10. Jang YY, Collector MI, Baylin SB, Diehl AM, Sharkis SJ et al. Hematopoietic stem cells convert into liver cells within days without fusion. Nat Cell Biol 2004; 6: 532-9.
11. Aliotta JM, Sanchez-Guijo FM, Dooner GJ, Johnson KW, Dooner MS, Greer KA, Greer D et al. Alteration of marrow cell gene expression, protein production, and engraftment into lung by lung-derived microvesicles: a novel mechanism for phenotype modulation. Stem Cells 2007;25: 2245-56.
12. Ratajczak J, Miekus K, Kucia M, Zhang J, Reca R, Dvorak P et al. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. Leukemia 2006;20: 847-56.
13. Imanishi D, Miyazaki Y, Yamasaki R, Sawayama Y, Taguchi J, Tsushima H et al. Donor-derived DNA in fingernails among recipients of allogeneic hematopoietic stem-cell transplants. Blood 2007;110: 2231-4.

14. Waterhouse M, Themeli M, Bertz H, Zoumbos N, Finke J, Spyridonidis A et al. Horizontal DNA transfer from donor to host cells as an alternative mechanism of epithelial chimerism after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 2010;17: 319-29.
15. Rutkowska J. and Olszewski WL, DNA transfer in bacteria and animals (humans). Ann Transplant 2006;11: 16-21.
16. Burghoff S, Ding Z, Gödecke S, Assmann A, Wirrwar A, Buchholz D et al. Horizontal gene transfer from human endothelial cells to rat cardiomyocytes after intracoronary transplantation. Cardiovasc Res 2008; 77: 534-43.
17. García-Olmo D, García-Olmo DC, Ontañón J, Martinez E, Vallejo M. Tumor DNA circulating in the plasma may play a role in metastasis. The hypotesis of the genometastasis. Histol Histopathol 1999; 14: 1159-1164.
18. Kirsch T, Woywodt A, Beese M, Wyss K, Park JK, Erdbruegger U et al. Engulfment of apoptotic cells by microvascular endothelial cells induces proinflammatory responses. Blood 2007;109: 2854-62.
19. Kleinclauss F, Perruche S, Cahn JY, Tiberghien P, Saas P et al. Administration of donor apoptotic cells: an alternative cell-based therapy to induce tolerance? Transplantation 2003;75: 43S-45S.
20. Tysnes BB, Bjerkvig R. Cancer initiation and progression: involvement of stem cells and the microenvironment. Biochim Biophys Acta 2007;1775: 283-97.
21. Halicka HD, Bedner E, Darzynkiewicz Z. Darzynkiewicz, Segregation of RNA and separate packaging of DNA and RNA in apoptotic bodies during apoptosis. Exp Cell Res 2000;260: 248-56.
22. Savill J, Fadok V. Corpse clearance defines the meaning of cell death. Nature 2000;407: 784-8.
23. Holmgren L, Szeles A, Rajnavölgyi E, Folkman J, Klein G, Ernberg I et al. Horizontal transfer of DNA by the uptake of apoptotic bodies. Blood 1999;93: 3956-3963.
24. Bergsmedh A, Szeles A, Henriksson M, Bratt A, Folkman MJ, Spetz AL et al. Horizontal transfer of oncogenes by the uptake of apoptotic bodies. Proc Nat Acad Sci 2001;98: 6407-6411.
25. Schubbert R, Renz D, Schmitz B, Doerfler W. Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. Proc Natl Acad Sci USA, 1997;94: 961-6.
26. Doerfler W, Hohlweg U, Müller K, Remus R, Heller H, Hertz J. Foreign DNA integration--perturbations of the genome--oncogenesis. Ann NY Acad Sci 2001; 945: 276-88.
27. Tysnes BB, Bjerkvig R. Cancer initiation and progression: Involvement of stem cells and the microenvironment. Biochim Biophys Acta 2007;1775: 283-297.
28. Partsalis, T., et al., Evidence of circulating donor genetic material in bone allotransplantation. Int J Mol Med, 2006. 17: p. 1151-5.
29. Partsalis T, Chan LY, Hurworth M, Willers C, Pavlos N, Kumta N et al. Horizontal transfer of tumor DNA to endothelial cells in vivo. Cell Death Differ 2009;16: 749-57.
30. Khan FM, Sy S, Louie P, Ugarte-Torres A, Berka N, Sinclair GD et al. Genomic instability after allogeneic hematopoietic cell transplantation is frequent in oral mucosa, particularly in patients with a history of chronic graft-versus-host disease, and rare in nasal mucosa. Blood 2010;116: 1803-6.
31. Themeli M, Petrikkos L, Waterhouse M, Bertz H, Lagadinou E, Zoumbos N et al., Alloreactive microenvironment after human hematopoietic cell transplantation induces genomic alterations in epithelium through an ROS-mediated mechanism: in vivo and in vitro study and implications to secondary neoplasia. Leukemia 2010;24: 536-43.